Journal of Advanced Veterinary Research
Volume 1 (2011) 119-126 **Original Research**

Evaluation of Subacute Toxicity Induced by Methoxychlor: The protective Effect of Ascorbic acid

Eman E. Elsharkawy*, Sharkawy A.A.
Departement of Fronsic Meicine and Toxicology, Faculty of veterinary Medicine, Assuit university, Egypt

(Recieved 27 Jun 2011/ Accepted 28 September 2011)

Abstract

The present study was designed to detect the effects of sublethal concentration of Methoxychlor (MXC) on hematological constituents, thrombocytic indices, liver and kidney damage biomarker and erythrocytic oxidative status. As well as the possible protective effect of ascorbic acid (vit-C) were tested in rats at subacute period under laboratory conditions. MXC was administered orally at a dose of 200mg/kg, twice weekly, to male rats, for one month. Co – administration of ascorbic acid at a dose of 1g\L drinking water was achieved. MXC caused a significant reduction in hematological constituents and thrombocytic indices. MXC induced a significant increase in the serum alkaline phosphatase (ALP), gamma glutamyle transferase (γ-GT), uric acid and creatinine, whereas decrease in total protein and albumin levels. With regards, to the results of oxidative status revealed that decrease in superoxide dismutase (SOD) and glutathione peroxidase (GSHPx) activities while increase in lipid peroxidation (LPO) components malondialdehyde (MDA). MXC plus vit-C treated group was significantly differed in most previous parameters than the MXC -treated group. These data, along with the determined changes suggest that MXC produce substantial systemic organ toxicity in rats during the period of a 30-days subacute exposure. The co- treatment with vit- C could ameliorate these toxic changes.

Keywords: Methoxychlor; ascorbic acid; hematological; thrombocytic; oxidative status

Introduction

Methoxychlor (MXC) a chlorinated hydrocarbon insecticide, is closely related to DDT. Methoxychlor is less toxic than DDT because of its shorter half-life in mammals and easier biodegradability (Bal, 1984). This advantage led it to its wide use to control a broad range of insects that attack fruits, vegetables, shade trees, home gardens, forage crops, and livestock in many countries (Reuber, 1980). However, recent investigations indicate that MXC may have an endocrine disrupting potential that mimics estrogenic activity and disturbs the endocrine system (Soto *et al*., 1995, Shelby *et al*., 1996, Bolger *et al*., 1998, Pickford and Morris, 1999, Laws *et al*., 2000). Moreover, Chapin *et al*. (1997) reported that MXC affected the immune system as well as the reproductive system in young rats when exposed during the in utero and postnatal period. A few toxicological studies have addressed the possible relationship between reproductive toxicity and exposure to chemicals that generate reactive oxygen species (ROS) (Perreault, 1997). It has been reported that methoxychlor undergoes hepatic microsomal monooxygenase mediated activation and the resultant reactive metabolites, possibly free radicals, bind covalently to microsomal components (Bulger *et al*, 1983). Antioxidants/ free radical scavengers and sulfhydryl containing compounds inhibit covalent binding of methoxychlor in human liver microsomes, suggesting that the reactive intermediates are free radicals (Bulger *et al*., 1989). It has also been reported that human cytochrome P-450 enzymes responsible for conversion of methoxychlor into its major metabolites, the mono-o-demethylated derivatives, including CYP1A2 play the predominant role in this reaction (Stresser and Kupfer, 1998). The ability of the cytochrome P-450 system to induce the production of reactive oxygen species in hepatic and other tissues has been reported (Bondy and Naderi, 1994). It has been shown that methoxychlor induce oxidative stress in the epididymal sperm of goat (Gangadharan *et al*., 2001). ROS are formed in both physiologic and pathologic conditions in mammalian tissues; due to their high reactivity, ROS may in-

*Corresponding author: Eman E. Elsharkawy
Address: Departement of Fronsic Meicine and Toxicology, Faculty of veterinary Medicine, Assuit university, Egypt
E-mail address: medicine1971@yahoo.com

teract with biomolecules inducing oxidative stress (Ochsendoerf, 1999). This oxidative stress is related to the antioxidant defense system, and an overall balance between pro-oxidants and antioxidants is required to maintain cellular homeostasis. Environmental contaminants have been reported to disturb the prooxidant and antioxidant balance of cells (Jamieson, 1989) and to result in generation of oxygen free radicals and ROS (Harris *et al*., 1996).

Some studies have shown that vitamin C is used in pesticide toxicity in the experimental studies (Altuntas *et al*., 2002, Yavuz *et al.*, 2004). Many insecticides are hydrophobic molecules, which bind extensively to biological membranes, especially to the phospholipids bilayers (Lee *et al*., 1991). Vitamin C is hydrophilic and a most important free radical scavenger in extracellular fluids, trapping radicals in the aqueous phase, and protecting biomembranes from peroxidative damage (Yavuz *et al*., 2004). The majority of reported MXC toxicity studies in literature have focused on reproductive effects of MXC ((Bolger *et al*., 1998; Pickford and Morris, 1999; Laws *et al*., 2000) and there are limited data on other possible toxic effects induced by MXC exposure. For this reason, the aim of this study was to determine the effect of subacute MXC exposure on the hematological system, hepatic and renal integrity and oxidative status of rats, and also to assess whether these effects can be ameliorated by co-treatment with vitamins C.

Materials and methods

Chemicals

Methoxychlor (1,1,1-trichloro-2,2-bis [methoxyphenyl] ethane, Approx 95% and L- ascorbic acid (vitamin C) were purchased from Sigma (St.Louis,Mo.,USA). MXC was dissolved in corn oil (1:100). All other chemicals were of the highest grade available commercially.

Animal and treatment

Sprague–Dawley rats, weighing 180–250 g, obtained from the Animal Laboratory House of Assiut University, Assiut, Egypt were used for the study. The animals were housed in plastic cages and allowed to adjust to the new environment for a week before starting the experiment. Rats were fed standard food pellets and tap water ad libitum. The rats were housed at 24-25 ^{o}C and in daily dark/light cycle. The design of the study was in accordance with the ethical guidelines prescribed by the Institution. Animals were randomly divided into four groups of seven animals each Group A: exposed to MXC at a dose of 200 mg\kg b.w, twice \ week, orally by stomach tube for one month. Group B: as pervious, plus were administered an ascorbic acid at a dose of 1g\L drinking water ad libitum, for month. Group C: were administered an ascorbic acid at a dose of 1g\L drinking water ad libitum, for month. Control group: were administered corn oil only and kept as control.

Hematological parameters

Hematological parameters [Red Blood Corpuscles (RBC), Hemoglobin (HB), Hematocrite (HCT), Red Blood cell distribution width (RDW), Mean Cell Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), White Blood Corpuscles (WBC) and Differential Leucocytic levels]. Thrombocytic indices [Total Platelet Count (PLT), Mean Platelet Volume (MPV), Total Platelet Crit (PCT) and Platelet Distribution Width (PDW)] were analyzed by automated parameter hematology analyzer (MICROS 60-Abx Diagnostics, Montpellier, France).

Biochemical analysis

Serum was used to determine total protein and albumin by colorimetric method according to Doumas (1971). Gamma glutamate transferase (γ-GT) was measured according to Tietz (1994). Alkaline phosphatase ((ALP) was measured according to Rec (1972). Serum was used to determined creatinine level according to Sies *et al.* (1985), uric acid according to Tietz (1990).

Determination of lipid peroxidation

Measurement of malondialdehyde (MDA) and 4-hydroxyalkenals (HAE) have been used as an indicator of lipid peroxidation. MDA and 4HNE were estimated by the method of Buege and Aust (1978). Briefly 200 ml aliquot of erythrocytes lysates (10% w/v in Tris–HCl buffer, 20 mM, pH 7.4) was transferred to 650 ll of 10.3 mM 1-methyl-

2-phenylindole in acetonitrile and vortex mixed. To assay MDA + 4HNE, 150 ml of 15.4 M methanesulfonic acid was added, vortexed and incubated at 45 0C for 40 min. To assay MDA alone, 150 ml of 37% HCl was added instead of methanesulfonic acid, vortexed, incubated at 45 ^{o}C for 60 min. After incubation, samples were kept on ice, centrifuged at 9500g for 5 min and absorbance was measured at 586 nm. The levels of MDA and 4HNE are expressed as nmol of reactive substance formed/min/mg protein.

Determination of superoxide dismutase (SOD) activity in erythrocyte lysates

Changes in erythrocyte Cu/Zn superoxide dismutase (Cu/Zn-SOD) activity were analysed using the Bioxytech SOD-525 spectrophotometric assay kit. Briefly, whole blood samples from control and treated rats were collected in sterile EDTA Vacutainer tubes and centrifuged at 4 ^{o}C and 3000g. The plasma was removed from the erythrocyte pellet and erythrocyte lysates were prepared according to the protocol described in the kit. Spectrophotometric assay of SOD activity was based on the enzyme's ability to inhibit superoxide-driven NADH oxidation. The rate of reaction was measured by recording to the change in the absorbance at 550 nm. The activity was expressed as units per gram protein in erythrocyte (Nebt, 1991).

Determination of glutathione peroxidase (GSHPx) activity in erythrocyte lysates

The activities and concentrations of (GSHPx) were determined spectrophotometrically using a commercially available kit. This procedure based on the method described by Chu *et al.* (1993). The activities of GSHPx were measured as the production of NADP+ by the activation of glutathione reductase (GR) on oxidized glutathione (GSSG) in the presence of NADPH. The absorbance determined at 340 nm and the activity was given as units per gram protein in erythrocyte.

Determination of protein

Protein concentrations were measured by the method of Bradford (1976), using bovine serum albumin as a standard. Protein concentration used in the concentration of SOD, GSHPx and MDA & HAE can be expressed as activity per mg of protein by dividing the units /ml of protein concentration.

Statistical analysis

Data are expressed as means ± SD. Statistical analysis was performed to compare treated groups with respective control groups using one-way analysis of variance (ANOVA), followed by the Duncan's multiple range test when appropriate. Values of $P < 0.05$ were considered statistically significant.

Results

Hematological parameters

A significant ($P < 0.05$) decrease in RBC (10^6 /mm^3), Hg (g/dl), HCT (%), RDW (%), MCV(um), MCH (pg) and MCHC (g/dl) was obtained in exposed groups MXC-treated group A and MXC plus vitC –treated group B in comparison with control rats (Table. 1). Total and differential leucocytic count (x10 ^{3} /mm^3) showed a significant ($P < 0.05$) decrease than control in both group A and B (Table. 2). MXC-treated group (A) and the MXC plus vitC –treated group (B) were significantly decreased in terms of PLT (x10 ^{3} /mm^3), MPV (um), PCT (%) and PDW (um) values than the control (Table. 3). However, the MXC plus vitC –treated group was significantly differed in most previous parameters than the MXC -treated group (Table 1, 2 and 3). No significance difference present between vitC –treated group and control in all investigated parameters.

Biochemical parameters

A significant ($P < 0.05$) reduction in total protein and albumin concentration (g\dl) was obtained in the serum of rates in group A and B than the control. A significant ($P < 0.05$) elevation in ALP and γ- GT concentration (U/I) was recorded in the serum of rates in A and B groups than the control. As well as, there was a significant ($P < 0.05$) increase of creatinine and uric acids levels (mg\dl) in the serum of tested rats was recorded in both groups A and B than control. Treatment with vit C resulted in a significant ($P < 0.05$) decrease in ALP, γ- GT, creatinine and uric acids levels whereas increase in total protein and albumin concentration

between group A and B (Table. 4).

Lipid peroxidation

The influence of subacute toxicity of MXC and Vit C administration on lipid peroxidation MDA and 4HAE levels is shown in Table 5. Treatment with MXC resulted in a significant increase ($p < 0.05$) in plasma lipid peroxidation as measured by the amount of MDA formed. However, treatment with vitC to MXC-treated animals led to a significant decrease of lipid peroxidation ($p < 0.05$) between group B and group A rats.

Erythrocytes SOD and GSHPx

Table. 5 shows the influence of MXC exposure and treatment with vitC on the activities of the enzymes SOD and GSHPx. Subacute exposure to MXC resulted in a state of injury and extensive oxidative damage in rats erythrocytes as manifested by the significant declines ($p < 0.05$) in SOD and GSHPx enzyme levels compared to control rats. In contrast, treatment with vitC resulted in a small but significant ($p < 0.0\ 5$) amelioration of in the activities of enzymes SOD and GSHPx.

Table 1. Effect of subacute exposure to MXC on the different hematological indices of rat

Group	RBCs $10^6/mm^3$	Hg g/dl	HCT %	RDW %	MCV µm	MCH pg	MCHC g/dl
A	7.50±0.83*bc	12.35±1.7*bc	35.70±5.3*bc	22.79±0.9*bc	45.43±1.2*bc	16.53±0.1*bc	35.06±2.7*bc
B	8.34±0.37*ac	13.99±0.7*ac	37.16±4.2*ac	24.86±0.3*ac	47.34±2.1*ac	17.80±0.4*ac	36.14±0.3*ac
C	8.91±0.59ab	15.31±0.3ab	38.36±0.6ab	22.19±0.8 ab	49.93±1.8ab	18.71±1.6ab	36.44±0.6 ab
Control	8.74±0.31	15.00±0.5	38.65±1.35	21.50±1.21	50.50±0.46	18.15±0.02	33.40±2.0

Data are expressed as mean ± S.D. of seven animals per group.*denotes $P < 0.05$ as compared to control group, a denotes $P < 0.05$ as compared to group A. b denotes $P < 0.05\ 05$ as compared to group B. c denotes $P < 0.05\ 05$ as compared to group C. (One- way ANOVA/Duncan).

Table 2. Effect of subacute exposure to MXC on total and differential leucocytic count ($x10^3/mm^3$) of rat

Group	WBCs Count	Lymphocytes Count	Granulocytes count	Monocytes Count
A	7.32 ±1.0*bc	4.17 ±0.87*bc	2.92±0.62*bc	1.00±0.33
B	9.67± 3.0*ac	5.25±2.46*ac	3.82±0 .59*ac	0.98±0.34
C	10.36±1.8ab	6.84 ±0.53ab	3.84± 0.28ab	0.81±0.10ab
Control	10.53±0.34	6.23 ±0.80	3.98± 0.80	0.80±0.05

Data are expressed as mean ± S.D. of seven animals per group.*denotes $P < 0.05$ as compared to control group, a denotes $P < 0.05$ as compared to group A. b denotes $P < 0.05\ 05$ as compared to group B. c denotes $P < 0.05\ 05$ as compared to group C. (One- way ANOVA/Duncan).

Table 3. Effect of subacute exposure to MXC on thrombocytic indices of rat

Group	PLT $10^3/mm^3$	MPV um	PDW um	PCT %
A	496.0±70.2*bc	5.52±0.34*bc	8.11±0.74*bc	0.24±0.02*bc
B	690.4±91.3*ac	6.20±0.22*ac	9.65±0.39*ac	0.42±0.04*ac
C	696.3±15.87ab	6.21±0.31ab	9.12±0.41ab	0.30± 0.01ab
Control	696.3±15.87	6.48±0.38	9.16± 0.17	0.30± 0.01

Data are expressed as mean ± S.D. of seven animals per group.*denotes $P < 0.05$ as compared to control group, a denotes $P < 0.05$ as compared to group A. b denotes $P < 0.05\ 05$ as compared to group B. c denotes $P < 0.05\ 05$ as compared to group C (One- way ANOVA/Duncan).

Table 4. Effect of subacute exposure to MXC on biochemical parameters of rat

	TP g\dl	Albumin g\dl	ALP U\l	γ- Gt U\ l	Uric acid mg\dl	Creatinine mg/dl
A	6.88±0.1* b	3.84±0.45*b	88.12±10*a	38.60±4.2* a	9.98±1.0*a	0.92±0.07*a
B	7.50±0.1*a	4.54±0.45*a	78.12±10*b	28.80±5.2*b	8.38±1.2*b	0.82±0.08*b
C	8.40±0.9*ab	5.62±0.91ab	56.88±21*ab	24.40±4.0ab	6.04±0.7 ab	0.56±0.05ab
Control	8.88±0.7	5.67±0.56	55.43±3	23.16±1.0	6.77±0.7	0.62±0.02

Data are expressed as means ± S.D. of five animals per group.*denotes P < 0.05 as compared to control group, a denotes P < 0.05 as compared to group A. b denotes P < 0.05 05 as compared to group B. c denotes P < 0.05 as compared to group C. (One- way ANOVA/Duncan).

Table 5. Effect of subacute exposure to MXC on the oxidative status of rat

	MDA & HAE (nmol/mg protein)	SOD (IU/mg protein)	GSHPx (IU/mg protein)
A	6.96±0.22*bc	0.56±0.07*bc	0.52±0.136*bc
B	5.00±0.16*ac	0.88±0.08*ac	0.75±0.237*ac
C	3.83±0.23ab	1.96±0.06ab	0.90±0.317ab
Control	3.50±0.31	1.63±0.15	0.98±0.243

Data are expressed as means ± S.D. of seven animals per group.*denotes P < 0.05 as compared to control group, a denotes P < 0.05 as compared to group A. b denotes P < 0.05 05 as compared to group B. c denotes P < 0.05 05 as compared to group C (One- way ANOVA/Duncan).

Discussion

Methoxychlor is considered of most important environmental estrogen. Methoxychlor is one of the endocrine disrupting chemicals (EDs) which are considered to act via receptors, including estrogen receptor (ER), and to modify the expression of estrogen-responsive genes (Golub *et al*., 2003). MXC, via its estrogenic metabolite HPTE, can have an ER- β antagonist effect (Gaido *et al*., 1999; Gaido *et al*., 2000, Waters *et al*., 2001). The attention to the effects of endocrine disruption has focused on reproductive system. However, long-term effects in a variety of systems relevant to future health could be altered when exogenous estrogens impinge on sensitive developmental periods. Some potential health impacts of estrogen disruption in adolescence include susceptibility to autoimmunity, heart disease, anemia, and osteoporosis (Golub *et al*., 2004). Moreover, it was reported that the toxicological effects like neurotoxicity, hepatotoxicity, reproductive, and metabolic disruption by EDs in different models continue to raise serious global concerns and are a subject of intensive investigations to decipher the molecular details of their malicious action (Tabb and Blumberg, 2006, Guillette, 2006). However, literatures on MXC induce hematological, hepatotoxicity and nephrotoxicity are limited, but the few available studies indicate that MXC affected the immune system as well as has toxicological effects like neurotoxicity, hepatotoxicity, and metabolic disruption (Chapin *et al*., 1997).

The present study revealed that MXC in subacute exposure was associated with significant decreases in RBCs count, Hg, HCT, RDW, MCV, MCH and MCHC values. A similar trend has been documented for EDs which have estrogenic activity like MXC; they induced depression of hematocrit value with a corresponding lowering of the RBC numbers and hemoglobin values during the treatment period (Golub *et al*., 2004). The obtained results suggested a slight anemic trend in MXC-treated rat, as indicated by decrease in hemoglobin concentration, hematocrit and red blood cells. The obtained anemia was microcytic as indicated by a decrease in MCV and it was normochromic as indicated by decrease in MCH. The decrease in the red blood cells may indicate a disruption of erythropoiesis or an increase in destruction of red blood cells. The administration of estrogens has been known to reduce erythropoiesis (Jilka *et al*., 1995, Suwalsky *et al*., 1998). The destruction of red blood cells is attributed to lipophilicity of most pesticides especially; organochlorine, makes lipid-rich membranes important targets of their interaction with living organisms. It has been suggested

that some effects directly related to their toxicity could be due to changes in membrane fluidity as a primary pesticide effect (Lopes *et al*., 1997). In vitro, human erythrocytes have a perturbing effect of the morphology through interaction of chlordane organochlorine insecticide with phospholipid classes located in the inner and outer monolayers of the erythrocyte cell membrane (Suwalsky *et al*., 2005). A significant depression in total WBC, lymphocytes and granulocytes counts was recorded after 4 weeks of exposure. Similar results were found in monkeys exposed to MXC for long time and they suggested the depression in total WBC resulted in the decrease in lymphocytes proliferation (Golub *et al*., 2004). MXC can induce depression of lymphoid proliferation via apoptosis in lymphoid organs. MXC is one of immunotoxic chemicals that have the potential to cause thymic atrophy through affect immature thymocytes and result in apoptosis (Koner *et al*.,1988, Takeuchi *et al*., 2002).

There is no previous literature discussed the effect of MXC on the thrombocytic index. The obtained results in this study revealed MXC-treated group was significantly decreased in terms of PLT, MPV, PCT and PDW values than the control. These results indicate MXC induce thrombocytopenia in exposed rats. The thrombocytopenia may develop due to antibody production against viral antigens attached to platelet surfaces or to nonspecific binding of antigen-antibody complexes to platelet surfaces. Drug and xenobiotic -induced thrombocytopenia has been reported in dogs, cats, and horses. One mechanism is marrow suppression of megakaryocytes or generalized marrow stem cell suppression after administration of estrogen, estrogen like compounds (Gibbins and Mahaut-Smith, 2004). Another mechanism is increased platelet destruction and consumption resulting in impair of platelet function. Numerous xenobitics reported to block platelet receptor binding or to change platelet membrane charge or permeability (Page *et al*., 2002). Quantitative platelet disorders have been reported in liver disease with or without coagulation protein deficiencies. In uremia associated with any form of renal disease, platelet adhesion and aggregation are decreased (Tkaczyk and Baj, 2002).

In this study the Liver damage was evaluated by the measurement of serum levels of the enzymes ALP and γ- GT. A significant increase in ALP and γ -GT levels was observed in the serum of MXC-treated rats than those of the control group. The values of serum proteins and albumin were significantly reduced in the same group. Following treatment with vit- C a significant improvement in the levels of ALP, γ- GT, total protein and albumin was seen. It was known MXC-induced hepatotoxicity where, liver is target site of its metabolism (Morgan and Hickenbottom, 1979). MXC undergoes hepatic microsomal monooxygenase (s)-mediated activation and the resultant reactive metabolites (possibly free radicals) bind covalently to microsomal components. It has been reported previously that during liver damage there is an observed decrease in antioxidant defenses in the liver (Suzuki *et al*., 1997). There were increase in both uric acid and creatinine serum levels in MXC – treated group in comparison with control. These results indicated that an impairment of bio-excretory function of the kidney, after subacute exposure to MXC for 4 weeks. In previous reports, documented that hepatotoxicity and nephrotoxicity induced after chronic exposure to MXC in different animals where, MXC in chronic intoxication of dogs at dosages of 2000 mg/ kg/ day in the diet led to high ALP and serum transaminase in 6 weeks. Swine showed kidney injury and uterine and mammary enlargement. Rabbits given 200 mg/ kg/ day orally died after four or five doses; autopsy findings included mild liver damage and nephrosis (Chen, 2002).

The by-products of oxygen metabolism initiate different subcellular outcomes. In the present study the effect of MXC on the oxidative status was studied by determination of SOD, GSHPx and MDA in erythrocytes. MXC has been shown to directly inhibit the activities of superoxide radical and glutathione peroxidase while increase of the activity of MDA lipid peroxidation. The results are in agreement with previous studies (Suzuki *et al*., 1997) where alterations in glutathione, total thiols, lipid peroxidation and changes in the activity of SOD and catalase in erythrocytes have been noted following exposure to pesticides. The mechanism of MXC-mediated oxidative stress is not clear but it has been shown to be mediated by the activation of microsomal monooxygenase, which is involved in the conversion of MXC into its reactive metabolites (Bulger *et al*., 1983). During this reaction, reactive metabolites, possibly free radicals, bind covalently to microsomal components (Bulger *et al*., 1989). Antioxidants/free radical scavengers and

sulfhydryl-containing compounds inhibit covalent binding of MXC in human liver microsomes, suggesting that the reactive intermediate is a free radical (Bulger *et al*., 1983). It has been shown that human cytochrome- P450 enzymes are responsible of the conversion of MXC into its major metabolites and CYP1A2 has been shown to play a predominant role in this reaction (Stresser and Kupfer, 1998). The ability of the cytochrome- P450 system to induce the production of ROS has been well documented (Bondy and Naderi, 1994). Superoxide dismutase is considered the first line of defense against deleterious effects of oxy-radicals in the cell by catalyzing the dismutation of superoxide radicals to hydrogen peroxide and molecular oxygen. The antioxidant enzyme peroxidase protects superoxide dismutase against inactivation by hydrogen peroxide. Reciprocally, the superoxide dismutase protects peroxidase against inhibition by superoxide anion (Latchoumycandane *et al*., 2002). Thus, balance of this enzyme system may be essential to eliminate superoxide anion and peroxides generated in erythrocytes. The reduction in the activities of antioxidant enzymes and the increase in lipid peroxidation could reflect an adverse effect of MXC on the antioxidant system in the erythrocytes. ROS cause damage to cells and other cytoplasmic organelle membrane structures through peroxidation of phospholipids, proteins, and nucleotides, thereby altering cell function.

Our results indicate that the MXC plus vit C – treated group was significantly differed in most previous parameters than the MXC -treated group. These findings reflected co-treatment of MXC-exposed rats with vitamins C ameliorated the toxic effects of MXC on rat. Antioxidant vitamins have a number of biological activities, including immune stimulation and alteration of the metabolic activities of carcinogens. These vitamins can also prevent genetic changes by inhibiting the DNA damage induced by reactive oxygen metabolites (Verma *et al*., 2007). Several experimental studies have shown that vitamins C and E could ameliorate pesticide toxicity (Altuntas *et al*., 2002, Yavuz *et al.*, 2004, Uzunhisarcikli *et al*., 2007). Vitamin C (ascorbic acid) is a well-known low molecular weight antioxidant that protects the cellular compartment from water-soluble oxygen nitrogen radicals (Jurczuk *et al*., 2007). It efficiently inhibits in vitro lipid peroxidation due to a combination of direct radical interception and interaction with α-tocopherol as a co-antioxidant (Verma *et al*., 2007). In conclusion, MXC caused subacute hematotoxicity, hepatotoxicity and nephrotoxicity. Vitamin C decreases MXC toxicity, but we cannot say Vitamin C protects completely.

References

Altuntas, I., Delibas, N., Sutcu, R., 2002. The effects of organophosphate insecticide methidathion on lipid peroxidation and anti-oxidant enzymes in rat erythrocytes: role of vitamins E and C. Human and Experimental Toxicology 21, 681–685.

Bal, H.S., 1984. Effect of methoxychlor on reproductive systems of the rat. Proceedings of the Society for Experimental Biology and Medicine 176 (2), 187–196.

Bolger, R., Wiese, T.E., Ervin, K., Nestich, S., Checovich, W., 1998. Rapid screening of environmental chemicals for estrogen receptor binding capacity. Environmental Health Perspectives 106, 551-557.

Bondy, S.C., Naderi, S., 1994. Contribution of hepatic cytochrome P450 systems to the generation of reactive oxygen species. Biochemical Pharmacology 48:155–9.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72, 248-54.

Buege, J.A., Aust, S.D., 1978. Microsomal lipid peroxidation. Methods in Enzymology 52, 306–310.

Bulger, W.H., Temple, J.E., Kupfer, D., 1983. Covalent binding of [14C] methoxychlor metabolite(s) to rat liver microsomal components. Toxicology and Applied Pharmacology 68, 367–74.

Bulger, W.H., Kupfer, D., 1989. Characteristics of monooxygenase-mediated covalent binding of methoxychlor in human and rat liver microsomes. Drug Metabolism and Disposition 17, 487–494.

Chapin, R.E., Harris, M.W., Davis, B.J., Ward, S.M., Wilson, R.E., Mauney, M.A., Lockhart, A.C., Smialowicz, R.J., Moser, V.C., Burka, L.T., Collins, B.J., 1997. The effects of perinatal/juvenile methoxychlor exposure on adult rat nervous, immune, and reproductive system function. Fundamental and Applied Toxicology 40, 138-157.

Chen, S., 2002. Modulation of aromatase activity and expression by environmental chemicals. Front Biosci. 7, 1712–1719.

Jamieson, D., 1989. Oxygen toxicity and reactive oxygen metabolites in mammals. Free Radical Biology and Medicine 7, 87-108.

Chu, F.F., Doroshow, J.H., Esworthy, R.S., 1993. Expression, characterization, and tissue distribution of a new cellular selenium-dependent glutathione peroxidase, GSHPx-GI. Journal of Biological Chemistry.5; 268 (4), 2571-2576.

Doumas, B., 1971. Biochemical determination of albumin concentration. Clinical Chemistry Acta 31, 87.

Gaido, K. W., Leonard, L. S., Maness, S. C., Hall, J. M., McDonnell, D. P., Saville, B., Safe, S. 1999. Differential interaction of the methoxychlor metabolite 2,2-bis-(phydroxyphenyl)-1,1,1-trichloroethane with estrogen receptors alpha and beta. Endocrinology 140, 5746-5753.

Gaido, K.W., Maness, S. C., McDonnell, D.P., Dehal, S.S., Kupfer, D., Safe, S., 2000. Interaction of methoxychlor and related compounds with estrogen receptor α and β, and androgen receptor: structure-activity studies. Molecular Pharmacology 58 (4), 852–858.

Gangadharan, B., Murugan, M.A., Mathur, P.P., 2001.Effect of methoxychlor on a Guillette. L.J., Jr. (2006). Endocrine disrupting contaminants-beyond the dogma. Environmental

Health Perspectives 114:9–12.

Gibbins, M.J., and Mahaut-Smith, P.M., 2004. Platelets and Megakaryocytes: Perspectives and Techniques. Published by Humana Pr Inc. ISBN is 1588291014.

Golub, M., Hogrefe, C., Germann, S., Lasley, B., Natarajan, K., Tarantal, A. F. 2003. Effects of exogenous estrogenic agents on pubertal growth and reproductive system maturation in female rhesus monkeys. Toxicological Science 74, 103-113.

Golub , M., Hogrefe , C ., Germann, S., Jerome, C., 2004. Endocrine disruption in adolescence: Immunologic, hematologic and bone effects in monkeys. ToxSci Advance Access 29.

Harris, C., Lee, E., Hiranruengchok, R., 1996. Characteristics of glutathione redox and antioxidant status in post implantation rat embryos: response to oxidative stress. Toxicologist 30, 2.

Jilka, R. L., Passeri, G., Girasole, G., Cooper, S., Abrams, J., Broxmeyer, H., Manolagas, S. C. 1995. Estrogen loss upregulates hematopoiesis in the mouse: a mediating role of IL-6. Experimental Hematology 23, 500-506.

Jurczuk, M., Brzoska, M.M., Moniuszko-Jakoniuk, J., 2007. Hepatic and renal concentrations of vitamins E and C in lead- and ethanol-exposed rats. An assessment of their involvement in the mechanisms of peroxidative damage. Food and Chemical Toxicology 45, 1478–1486.

Koner, B.C., Banerjee, B.D, Ray, A., 1988. Organochlorine pesticideinduced oxidative stress and immune suppression in rats. Indian Journal of Experimental Biology 36, 395–398.

Latchoumycandane, C., Chitra, K.C., Mathur, P.P., 2002. The effect of methoxychlor on the epididymal antioxidant system of adult rats. Reproductive Toxicology 16 (2), 161–172.

Laws, S.C., Carey, S.A., Ferrell, J.M., Bodman, G.J., Cooper, R.L., 2000. Estrogenic activity of octylphenol, nonylphenol, bisphenol A and methoxychlor in rats. Toxicological Science 54, 154–167.

Lee, A., East, J., Balgaug, P., 1991. Interactions of insecticides with biological membranes. Journal of Pesticide Science 32, 317–327.

Lopes, V.I.C.F., Antunes-Madeira, M.C., Madeira, V.M.C., 1997. Effects of methylparathion on membrane fluidity and its implications for the mechanism of toxicity, Toxicology In Vitro 11, 337– 345.

Morgan, M., and Hickenbottom,P., 1979. Comparison of selected parameters for monitoring methoxychlor-induced hepatotoxicity. Bulletin of Environmental Contamination and Toxicology. 1, 275-280.

Nebot, C., Moutet, M., Huet, P., Xu, J.Z., Yadan, J.C., Chaudiere, J., 1991. Spectrophotometric Assay of Superoxide Dismutase activity based on activated autoxidation of a tetracyclic catechol. Analytical biochemistry.214, 442-451.

Ochsendoerf, F.R., (1999). Infections in the male genital tract and reactive oxygen species. Hum Reprod Update. 5, 399–420.

Page, C., Fuster, V., Gresele, P., Vermylen, J., 2002. Platelets in Thrombotic and Non-Thrombotic Disorders: Pathophysiology, Pharmacology and Therapeutics. Published by Cambridge Univ Pr. ISBN is 052180261X

Perreault, S.D., 1997.The mature spermatozoan as a target for reproductive toxicants. In: Comprehensive Toxicology: Reproductive and Endocrine Toxicology. Vol. 10. (ed. Kim Bokelheide, Robert E. Chapin, Patricia B. Hoyer and Craig Harris. 165–91.

Pickford, D.B., Morris, D., 1999. Effect of endocrine-disrupting cintaminants on amphibian oogenesis: methoxychlor inhibits progesterone-induced maturation of Xenopus laevis oocytes in vitro. Environmental Health Perspective 107, 285- 292.

Rec, G. S., 1972: Determination of alkaline phosphatase. Journal of Clinical Chemistry and Clinical Biochemistry 10; 82.

Reuber, M.D., 1980. Carcinogenicity and toxicity of methoxychlor. Environmental Health Perspective 36, 205-219.

Sies, G., Henny, J., Schiele, F. and Young, D., 1985. Interpretation of clinical laboratory tests. Biomedical publication. pp. 220 – 234.

Shelby, M.D., Newbold, R.R., Tully, D.B., Chae, K., Davis, V.L., 1996. Assessing environmental chemicals for estrogenicity using a combination of in vitro and in vivo assays. Environmental Health Perspectives 104, 1296-1300.

Soto, A.M., Sonnenschein, C., Chung, K.L., Fernandez, M.F., Olea, N., Serrano, F.O., 1995. The E-SCREEN assay as a tool to identify estrogens: an update on estrogenic environmental pollutants. Environmental Health Perspectives 103, 113-122.

Stresser, D.M., Kupfer, D., 1998. Human cytochrome P450 catalyzed conversion of the proestrogenic pesticide methoxychlor into an estrogen. Role of CYP2C19 and CYP 1A2 in O-demethylation. Drug Metabolism and Disposition 26:868–74.

Suwalsky, M., Rodrı´guez, C., Villena, F., Aguilar, F., Sotomayor, C.P., 1998. The organochlorine pesticide lindane interacts with the human erythrocyte membrane. Pesticide Biochemistry and Physiology 62, 87–95.

Suwalsky,M., Rodrı´guez ,C., Villena ,F.,Sotomayor, C.P., 2005. Human erythrocytes are affected by the organochloride insecticide chlordane. Food and Chemical Toxicology 43, 647–654.

Suzuki, T., Komatsu, M., Isono, H., 1997. Cytotoxicity of organochlorine pesticides and lipid peroxidation in isolated rat hepatocytes. Biol Pharm Bull, 203:271–274.

Tabb, M.M., Blumberg, B., 2006. New modes of action for endocrine- disrupting chemicals. Molecular Endocrinology 20, 475–482.

Tietz, N.W., 1990. Clinical guide to laboratory tests. 2nd ed. Philadelphia: W13 Souners:pp. 566.

Tietz, N.W., 1994. Textbook of Clinical Chemistry .2nd ed. W. B. Sonuners Co.philadelphia.pp.851-860.

Tkaczyk, M., Baj, Z., 2002. Surface markers of platelet function in idiopathic nephrotic syndrome in children. Pediatric Nephrology 17, 673–677.

Takeuchi, T., Kosaka, T., Hayashi, K., Takeda, M., Yoshida, T., Fujisawa, H., Teramoto, S., Maita, K., Harada, T., 2002. Thymic atrophy induced by methoxychlor in rat pups. Toxicology Letters 135, 199-207.

Uzunhisarcikli, M., Kalender, Y., Dirican, K., Kalender, S., Ogutcu, A., Buyukkomurcu, F., 2007. Acute, subacute and subchronic administration of methyl parathioninduced testicular damage in male rats and protective role of vitamins C and E. Pesticide Biochemistry and Physiology 87, 115–122.

Verma, R.S., Mehta, A., Srivastava, N., 2007. In vivo chlorpyrifos induced oxidative stres: attenuation by antioxidant vitamins. Pesticide Biochemistry and Physiology 88, 191–196.

Waters, K. M., Safe, S., and Gaido, K. W. 2001. Differential gene expression in response to methoxychlor and estradiol through ERalpha, ERbeta, and AR in reproductive tissues of female mice. Toxicological Science 63, 47-56.

Yavuz, T., Delibas, N., Yildirim, B., Altuntas, I., Candir, O., Cora, A., Karaman, N., Ibrisim, E., Kutsal, A., 2004. Vascular wall damage in rats induced by methidathion and ameliorating effect of vitamins E and C. Archive of Toxicology 78, 655–659.